TITLE OF THE INVENTION

POLYPEPTIDES FOR INDUCING A PROTECTIVE IMMUNE RESPONSE AGAINST *STAPHYLOCOCCUS AUREUS*

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of U.S. Provisional Application No. 60/548,660, filed February 27, 2004, which is hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

The references cited throughout the present application are not admitted to be prior art to the claimed invention.

Staphylococcus aureus is a pathogen responsible for a wide range of diseases and conditions. Examples of diseases and conditions caused by *S. aureus* include bacteremia, infective endocarditis, folliculitis, furuncle, carbuncle, impetigo, bullous impetigo, cellulitis, botryomyosis, toxic shock syndrome, scalded skin syndrome, central nervous system infections, infective and inflammatory eye disease, osteomyletitis and other infections of joints and bones, and respiratory tract infections. (*The Staphylococci in Human Disease*, Crossley and Archer (eds.), Churchill Livingstone Inc. 1997.)

Immunological based strategies can be employed to control *S. aureus* infections and the spread of *S. aureus*. Immunological based strategies include passive and active immunization. Passive immunization employs immunoglobulins targeting *S. aureus*. Active immunization induces immune responses against *S. aureus*.

Potential *S. aureus* vaccines target *S. aureus* polysaccharides and polypeptides. Targeting can be achieved using suitable *S. aureus* polysaccharides or polypeptides as vaccine components. Examples of polysaccharides that may be employed as possible vaccine components include *S. aureus* type 5 and type 8 capsular polysaccharides. (*Shinefield et al., N. Eng. J. Med. 346*:491-496, 2002.) Examples of polypeptides that may be employed as possible vaccine components include collagen adhesin, fibrinogen binding proteins, and clumping factor. (Mamo *et al., FEMS Immunology and Medical Microbiology 10*:47-54, 1994, Nilsson *et al., J. Clin. Invest. 101*:2640-2649, 1998, Josefsson *et al., The Journal of Infectious Diseases 184*:1572-1580, 2001.)

Information concerning *S. aureus* polypeptide sequences has been obtained from sequencing the *S. aureus* genome. (Kuroda *et al., Lancet 357*:1225-1240, 2001, Baba *et al., Lancet 359*:1819-1827, 2000, Kunsch *et al.,* European Patent Publication EP 0 786 519, published July 30, 1997.) To some extent bioinformatics has been employed in efforts to

characterize polypeptide sequences obtained from genome sequencing. (Kunsch *et al.*, European Patent Publication EP 0 786 519, published July 30, 1997.)

Techniques such as those involving display technology and sera from infected patients have been used in an effort to identify genes coding for potential antigens. (Foster *et al.*, International Publication Number WO 01/98499, published December 27, 2001, Meinke *et al.*, International Publication Number WO 02/059148, published August 1, 2002, Etz *et al.*, *PNAS* 99:6573-6578, 2002.)

SUMMARY OF THE INVENTION

The present invention features polypeptides comprising an amino acid sequence structurally related to SEQ ID NO: 1 and uses of such polypeptides. SEQ ID NO: 1 is a truncated derivative of a full length *S. aureus* polypeptide. The full-length polypeptide is referred to herein as full length "ORF0594". A His-tagged derivative of SEQ ID NO: 1 was found to produce a protective immune response against *S. aureus*.

Reference to "protective" immunity or immune response indicates a detectable level of protection against *S. aureus* infection. The level of protection can be assessed using animal models such as those described herein.

Thus, a first aspect of the present invention describes a polypeptide immunogen consisting essentially of an amino acid sequence at least 90% identical to SEQ ID NO: 1. Reference to consisting essentially of an amino acid sequence at least 90% identical to SEQ ID NO: 1 indicates that a SEQ ID NO: 1 related region is present and additional polypeptide regions up to about 100 amino acids also may be present.

A SEQ ID NO: 1 related region has at least about 90% sequence identity to SEQ ID NO: 1. Percent identity (also referred to as percent identical) to a reference sequence is determined by aligning the polypeptide sequence with the reference sequence and determining the number of identical amino acids in the corresponding regions. This number is divided by the total number of amino acids in the reference sequence (*e.g.*, SEQ ID NO: 1) and then multiplied by 100 and rounded to the nearest whole number.

Reference to "immunogen" indicates the ability to produce a protective immune response.

Another aspect of the present invention describes an immunogen comprising a polypeptide that provides protective immunity against *S. aureus* and one or more additional regions or moieties covalently joined to the polypeptide at the carboxyl terminus or amino terminus, wherein each region or moiety is independently selected from a region or moiety

having at least one of the following properties: enhances the immune response, facilitates purification, or facilitates polypeptide stability.

Reference to "additional region or moiety" indicates a region or moiety different from an ORF0594 region. The additional region or moiety can be, for example, an additional polypeptide region or a non-peptide region.

Another aspect of the present invention describes a composition able to induce protective immunity against *S. aureus* in a patient. The composition comprises a pharmaceutically acceptable carrier and an immunologically effective amount of a polypeptide that provides protective immunity against *S. aureus* or an immunogen containing the polypeptide.

An immunologically effective amount is an amount sufficient to provide protective immunity against *S. aureus* infection. The amount should be sufficient to significantly prevent the likelihood or severity of a *S. aureus* infection.

Another aspect of the present invention describes a nucleic acid comprising a recombinant gene encoding a polypeptide that provides protective immunity against *S. aureus*. A recombinant gene contains recombinant nucleic acid encoding a polypeptide along with regulatory elements for proper transcription and processing (which may include translational and post translational elements). The recombinant gene can exist independent of a host genome or can be part of a host genome.

A recombinant nucleic acid is nucleic acid that by virtue of its sequence and/or form does not occur in nature. Examples of recombinant nucleic acid include purified nucleic acid, two or more nucleic acid regions combined together that provides a different nucleic acid than found in nature, and the absence of one or more nucleic acid regions (*e.g.*, upstream or downstream regions) that are naturally associated with each other.

Another aspect of the present invention describes a recombinant cell. The cell comprises a recombinant gene encoding a polypeptide that provides protective immunity against *S. aureus*.

Another aspect of the present invention describes a method of making a polypeptide that provides protective immunity against *S. aureus*. The method involves growing a recombinant cell containing recombinant nucleic acid encoding the polypeptide and purifying the polypeptide.

Another aspect of the present invention describes a polypeptide that provides protective immunity against *S. aureus* made by a process comprising the steps of growing the recombinant cell containing recombinant nucleic acid encoding the polypeptide in a host and purifying the polypeptide. Different host cells can be employed.

Another aspect of the present invention describes a method of inducing a protective immune response in a patient against *S. aureus*. The method comprises the step of administering to the patient an immunologically effective amount of an immunogen providing protective immunity.

Unless particular terms are mutually exclusive, reference to "or" indicates either or both possibilities. Occasionally phrases such as "and/or" are used to highlight either or both possibilities.

Reference to open-ended terms such as "comprises" allows for additional elements or steps. Occasionally phrases such as "one or more" are used with or without open-ended terms to highlight the possibility of additional elements or steps.

Unless explicitly stated reference to terms such as "a" or "an" is not limited to one. For example, "a cell" does not exclude "cells". Occasionally phrases such as one or more are used to highlight the possible presence of a plurality.

Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the amino acid sequence of ORF0594 (SEQ ID NO: 3). Signal cleavage cite (AA) is marked in italics, LPXTG is marked in bold, and non identical repeats are alternatively underlined or double underlined.

Figure 2 illustrates a sequence comparison between SEQ ID NOs: 2 and 3. SEQ ID NO: 2 is a His-tagged derivative of SEQ ID NO: 1. The region of SEQ ID NO: 3 providing SEQ ID NO: 1 is shown in bold.

Figure 3 illustrates survival data from 2 experiments using a SEQ ID NO:2 polypeptide (SEQ 2) in aluminum hydroxyphosphate adjuvant (AHP). A positive control SEQ 4 (SEQ ID NO: 4) was present in Exp. 1.

Figure 4 illustrates the amino acid sequence of SEQ ID NO: 4.

Figure 5 illustrates a nucleic acid sequence (SEQ ID NO: 5) encoding SEQ ID NO: 2. The bold region represents the start/stop -added during cloning. The underlined region represents the SEQ ID NO: 1 encoding region. The region not underlined represents the N-term His tag vector sequence.

DETAILED DESCRIPTION OF THE INVENTION

SEQ ID NO: 1 is identified herein as a sufficient polypeptide region to provide protective immunity against *S. aureus* infection. The ability of polypeptides structurally related to SEQ ID NO: 1 to provide protective immunity against *S. aureus* infection is illustrated using a His-Tag derivative of SEQ ID NO: 1. (See Examples *infra.*)

ORF0594 belongs to the LPXTG family of staphylococcal surface proteins. Sequence analysis of SEQ ID NO: 3 revealed the presence of several imperfect repeats at the carboxyl terminal end (Figure 1). SEQ ID NO: 1 was designed to encompass an entire non-identical repeat and a portion of the flanking sequences so that a suspected coil-coil structure could be maintained.

Figure 2 illustrates the sequences of SEQ ID NOs: 1, 2, and 3. SEQ ID NO: 1 is illustrated in Figure 2 as the bolded region of SEQ ID NO: 3. SEQ ID NO: 2 is an amino His-tag derivative of SEQ ID NO: 1. The His-tag facilitates polypeptide purification and identification.

SEQ ID NO: 1 Related Polypeptides

A polypeptide region structurally related to SEQ ID NO: 1 contains an amino acid identity of at least 90% to SEQ ID NO: 1. Polypeptides containing a region structurally related to SEQ ID NO: 1 can be designed based on the guidance provided herein to obtain polypeptides protective against *S. aureus.*

Polypeptides consisting essentially of a region structurally related to SEQ ID NO: 1 can also contain additional polypeptide regions that may or may not be related to ORF0594. ORF0594 related polypeptides are polypeptides having at least about 90% sequence identity to a corresponding region of a naturally occurring ORF0594. A reference ORF0594 is illustrated in Figure 1 (SEQ ID NO: 3).

Additional regions can be at the carboxyl or amino terminus of the SEQ ID NO: 1 related region. In different embodiments 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, up to 25 or up to 50 additional amino acids are present; an amino terminus methionine is present; and/or the additional amino acids are ORF0594 related polypeptide regions.

Using SEQ ID NO: 1 as a frame of reference, alterations can be made taking into account the known properties of amino acids. Alterations include one or more amino acid additions, deletions, and/or substitutions. The overall effect of different alterations can be evaluated using techniques described herein to confirm the ability of a particular polypeptide to provide protective immunity.

Generally, in substituting different amino acids to retain activity it is preferable to exchange amino acids having similar properties. Factors that can be taken into account for an amino acid substitution include amino acid size, charge, polarity, and hydrophobicity. The effect of different amino acid R-groups on amino acid properties are well known in the art. (See, for example, Ausubel, *Current Protocols in Molecular Biology,* John Wiley, 1987-2002, Appendix 1C.)

In exchanging amino acids to maintain activity, the replacement amino acid should have one or more similar properties such as approximately the same charge and/or size and/or polarity and/or hydrophobicity. For example, substituting valine for leucine, arginine for lysine, and asparagine for glutamine are good candidates for not causing a change in polypeptide functioning.

Alterations to achieve a particular purpose include those designed to facilitate production or efficacy of the polypeptide; or cloning of the encoded nucleic acid. Polypeptide production can be facilitated through the use of an initiation codon (*e.g.,* coding for methionine) suitable for recombinant expression. The methionine may be later removed during cellular processing. Cloning can be facilitated by, for example, the introduction of restriction sites which can be accompanied by amino acid additions or changes.

Efficacy of a polypeptide to induce an immune response can be enhanced through epitope enhancement. Epitope enhancement can be performed using different techniques such as those involving alteration of anchor residues to improve peptide affinity for MHC molecules and those increasing affinity of the peptide-MHC complex for a T-cell receptor. (Berzofsky *et al., Nature Review 1*:209-219, 2001.)

A polypeptide region at least about 90% identical to SEQ ID NO: 1 contains up to about 19 amino acid alterations from SEQ ID NO: 1. In different embodiments, the SEQ ID NO: 1 related polypeptide is at least 90%, at least 95%, or at least 99% identical to SEQ ID NO: 1; or differs from SEQ ID NO: 1 by 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 amino acid alterations. Each amino acid alteration is independently an addition, deletion or substitution.

Longer length derivatives can be produced, for example, taking into account additional sequence information provided in SEQ ID NO: 3 or other naturally occurring proteins corresponding to SEQ ID NO: 3.

Preferably, the polypeptide is a purified polypeptide. A "purified polypeptide" is present in an environment lacking one or more other polypeptides with which it is naturally associated and/or is represented by at least about 10% of the total protein present. In different

embodiments, the purified polypeptide represents at least about 50%, at least about 75%, or at least about 95% of the total protein in a sample or preparation.

In an embodiment, the polypeptide is "substantially purified". A substantially purified polypeptide is present in an environment lacking all, or most, other polypeptides with which the polypeptide is naturally associated. For example, a substantially purified *S. aureus* polypeptide is present in an environment lacking all, or most, other *S. aureus* polypeptides. An environment can be, for example, a sample or preparation.

Reference to "purified" or "substantially purified" does not require a polypeptide to undergo any purification and may include, for example, a chemically synthesized polypeptide that has not been purified.

Polypeptide stability can be enhanced by modifying the polypeptide carboxyl or amino terminus. Examples of possible modifications include amino terminus protecting groups such as acetyl, propyl, succinyl, benzyl, benzyloxycarbonyl or *t*-butyloxycarbonyl; and carboxyl terminus protecting groups such as amide, methylamide, and ethylamide.

An embodiment of the present invention describes an immunogen consisting of a protective polypeptide and one or more additional regions or moieties covalently joined to the polypeptide at the carboxyl terminus or amino terminus. Each region or moiety should be independently selected from a region or moiety having at least one of the following properties: enhances the immune response, facilitates purification, or facilitates polypeptide stability. Polypeptide stability can be enhanced, for example, using groups such as polyethylene glycol that may be present on the amino or carboxyl terminus.

Polypeptide purification can be enhanced by adding a group to the carboxyl or amino terminus to facilitate purification. Examples of groups that can be used to facilitate purification include polypeptides providing affinity tags. Examples of affinity tags include a six-histidine tag, trpE, glutathione and maltose-binding protein.

The ability of a polypeptide to produce an immune response can be enhanced using groups that generally enhance an immune response. Examples of groups that can be joined to a polypeptide to enhance an immune response against the polypeptide include cytokines such as IL-2. (Buchan *et al.*, 2000. *Molecular Immunology 37*:545-552.)

Polypeptide Production

Polypeptides can be produced using standard techniques including those involving chemical synthesis and those involving purification from a cell producing the polypeptide. Techniques for chemical synthesis of polypeptides are well known in the art. (See *e.g.*, Vincent, *Peptide and Protein Drug Delivery*, New York, N.Y., Decker, 1990.)

Techniques for polypeptide purification from a cell are illustrated in the Examples provided below. Additional examples of purification techniques are well known in the art. (See for example, Ausubel, *Current Protocols in Molecular Biology,* John Wiley, 1987-2002.)

Obtaining polypeptides from a cell is facilitated using recombinant nucleic acid techniques to produce the polypeptide. Recombinant nucleic acid techniques for producing a polypeptide involve introducing, or producing, a recombinant gene encoding the polypeptide in a cell and expressing the polypeptide.

A recombinant gene contains nucleic acid encoding a polypeptide along with regulatory elements for polypeptide expression. The recombinant gene can be present in a cellular genome or can be part of an expression vector.

The regulatory elements that may be present as part of a recombinant gene include those naturally associated with the polypeptide encoding sequence and exogenous regulatory elements not naturally associated with the polypeptide encoding sequence. Exogenous regulatory elements such as an exogenous promoter can be useful for expressing a recombinant gene in a particular host or increasing the level of expression. Generally, the regulatory elements that are present in a recombinant gene include a transcriptional promoter, a ribosome binding site, a terminator, and an optionally present operator. A preferred element for processing in eukaryotic cells is a polyadenylation signal.

Expression of a recombinant gene in a cell is facilitated through the use of an expression vector. Preferably, an expression vector in addition to a recombinant gene also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high copy number. Examples of expression vectors are cloning vectors, modified cloning vectors, specifically designed plasmids and viruses.

Due to the degeneracy of the genetic code, a large number of different encoding nucleic acid sequences can be used to code for a particular polypeptide. The degeneracy of the genetic code arises because almost all amino acids are encoded by different combinations of nucleotide triplets or "codons". Amino acids are encoded by codons as follows:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU
C=Cys=Cysteine: codons UGC, UGU
D=Asp=Aspartic acid: codons GAC, GAU
E=Glu=Glutamic acid: codons GAA, GAG
F=Phe=Phenylalanine: codons UUC, UUU
G=Gly=Glycine: codons GGA, GGC, GGG, GGU
H=His=Histidine: codons CAC, CAU

I=Ile=Isoleucine: codons AUA, AUC, AUU
K=Lys=Lysine: codons AAA, AAG
L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU
M=Met=Methionine: codon AUG
N=Asn=Asparagine: codons AAC, AAU
P=Pro=Proline: codons CCA, CCC, CCG, CCU
Q=Gln=Glutamine: codons CAA, CAG
R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU
S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU
T=Thr=Threonine: codons ACA, ACC, ACG, ACU
V=Val=Valine: codons GUA, GUC, GUG, GUU
W=Trp=Tryptophan: codon UGG
Y=Tyr=Tyrosine: codons UAC, UAU

Suitable cells for recombinant nucleic acid expression of ORF0594 related polypeptides are prokaryotes and eukaryotes. Examples of prokaryotic cells include *E. coli*; members of the *Staphylococcus* genus, such as *S. aureus*; members of the *Lactobacillus* genus, such as *L. plantarum*; members of the *Lactococcus* genus, such as *L. lactis*; and members of the *Bacillus* genus, such as *B. subtilis*. Examples of eukaryotic cells include mammalian cells; insect cells; yeast cells such as members of the *Saccharomyces* genus (*e.g., S. cerevisiae*), members of the *Pichia* genus (*e.g., P. pastoris*), members of the *Hansenula* genus (*e.g., H. polymorpha*), members of the *Kluyveromyces* genus (*e.g., K. lactis* or *K. fragilis*) and members of the *Schizosaccharomyces* genus (*e.g., S. pombe*).

Techniques for recombinant gene production, introduction into a cell, and recombinant gene expression are well known in the art. Examples of such techniques are provided in references such as Ausubel, *Current Protocols in Molecular Biology,* John Wiley, 1987-2002, and Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual,* 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

If desired, expression in a particular host can be enhanced through codon optimization. Codon optimization includes use of more preferred codons. Techniques for codon optimization in different hosts are well known in the art.

Depending upon the host used for expression, ORF0594 related polypeptides may contain post translational modifications. Reference to "polypeptide" or an "amino acid" sequence of a polypeptide includes polypeptides containing one or more amino acids having a structure of a post-translational modification from a host cell, such as a yeast host.

For example, in *S. cerevisiae*, the nature of the penultimate amino acid appears to determine whether the N-terminal methionine is removed. Furthermore, the nature of the penultimate amino acid also determines whether the N-terminal amino acid is N^{α}-acetylated (Huang *et al.*, *Biochemistry 26*: (1987), 8242-8246, 1987). Thus, within the scope of this invention, the ORF0594-related polypeptide may have an N^{α}-acetylated N-terminus and the N-terminal methionine may be removed, depending on which amino acid is in the penultimate position.

In addition, if the ORF0594-related polypeptide is targeted for secretion due to the presence of a secretory leader (*e.g.*, signal peptide), the protein may be modified by N-linked or O-linked glycosylation. (Kukuruzinska *et al.*, *Ann. Rev. Biochem. 56*:915-944, 1987.)

Adjuvants

Adjuvants are substances that can assist an immunogen in producing an immune response. Adjuvants can function by different mechanisms such as one or more of the following: increasing the antigen biologic or immunologic half-life; improving antigen delivery to antigen-presenting cells; improving antigen processing and presentation by antigen-presenting cells; and inducing production of immunomodulatory cytokines. (Vogel, *Clinical Infectious Diseases 30*(suppl. 3):S266-270, 2000.)

A variety of different types of adjuvants can be employed to assist in the production of an immune response. Examples of particular adjuvants include aluminum hydroxide, aluminum phosphate, or other salts of aluminum, calcium phosphate, DNA CpG motifs, monophosphoryl lipid A, cholera toxin, *E. coli* heat-labile toxin, pertussis toxin, muramyl dipeptide, Freund's incomplete adjuvant, MF59, SAF, immunostimulatory complexes, liposomes, biodegradable microspheres, saponins, nonionic block copolymers, muramyl peptide analogues, polyphosphazene, synthetic polynucleotides, IFN-γ, IL-2 and IL-12. (Vogel *Clinical Infectious Diseases 30*(suppl 3):S266-270, 2000, Klein *et al., Journal of Pharmaceutical Sciences 89*:311-321, 2000.)

Patients For Inducing Protective Immunity

A "patient" refers to a mammal capable of being infected with *S. aureus*. A patient can be treated prophylactically or therapeutically. Prophylactic treatment provides sufficient protective immunity to reduce the likelihood, or severity, of a *S. aureus* infection. Therapeutic treatment can be performed to reduce the severity of a *S. aureus* infection.

Prophylactic treatment can be performed using a vaccine containing an immunogen described herein. Such treatment is preferably performed on a human. Vaccines can

be administered to the general population or to those persons at an increased risk of *S. aureus* infection.

Persons with an increased risk of *S. aureus* infection include health care workers; hospital patients; patients with a weakened immune system; patients undergoing surgery; patients receiving foreign body implants, such a catheter or a vascular device; patients facing therapy leading to a weakened immunity; and persons in professions having an increased risk of burn or wound injury. (*The Staphylococci in Human Disease*, Crossley and Archer (ed.), Churchill Livingstone Inc. 1997.)

Non-human patients that can be infected with *S. aureus* include cows, pigs, sheep, goats, rabbits, horses, dogs, cats and mice. Treatment of non-human patients is useful in protecting pets and livestock, and in evaluating the efficacy of a particular treatment.

Combination Vaccines

ORF0594 related polypeptides providing protective immunity can be used alone, or in combination with other immunogens, to induce an immune response. Additional immunogens that may be present include: one or more additional *S. aureus* immunogens, such as those referenced in the Background of the Invention *supra*; one or more immunogens targeting one or more other *Staphylococcus* organisms such as *S. epidermidis, S. haemolyticus, S. warneri,* or *S. lugunensis*; and one or more immunogens targeting other infectious organisms.

Animal Model System

An animal model system was used to evaluate the efficacy of a polypeptide to produce a protective immune response against *Staphylococcus*. Two obstacles encountered in setting up a protective animal model were: (1) very high challenge dose needed to overcome innate immunity and (2) death rate too fast to detect a protective response. Specifically, after bacterial challenge mice succumbed to infection within 24 hours which did not provide sufficient time for the specific immune responses to resolve the infection. If the dose was lowered both control and immunized mice survived the infection.

These obstacles were addressed by using a slow kinetics lethality model involving *S. aureus* prepared from cells in stationary phase, appropriately titrated, and intravenously administered. This slow kinetics of death provides sufficient time for the specific immune defense to fight off the bacterial infection (*e.g.,* 10 days rather 24 hours).

S. aureus cells in stationary phase can be obtained from cells grown on solid medium. They can also be obtained from liquid, however the results with cells grown on solid media were more reproducible. Cells can conveniently be grown overnight on solid medium.

For example, *S. aureus* can be grown from about 18 to about 24 hours under conditions where the doubling time is about 20-30 minutes.

S. aureus can be isolated from solid or liquid medium using standard techniques to maintain *Staphylococcus* potency. Isolated *Staphylococcus* can be stored, for example, at -70°C as a washed high density suspension (> 10^9 colony forming units (CFU)/mL) in phosphate buffered saline containing glycerol.

The *Staphylococcus* challenge should have a potency providing about 80 to 90% death in an animal model over a period of about 7 to 10 days starting on the first or second day. Titration experiments can be performed using animal models to monitor the potency of the stored *Staphylococcus* inoculum. The titration experiments can be performed about one to two weeks prior to an inoculation experiment.

Initial potency for titration experiments can be based on previous experiments. For *S. aureus* and the animal model strain Becker a suitable potency was generally found in the range of 5 x 10^8 to 8 x 10^8 CFU/ml.

Administration

Immunogens can be formulated and administered to a patient using the guidance provided herein along with techniques well known in the art. Guidelines for pharmaceutical administration in general are provided in, for example, *Vaccines* Eds. Plotkin and Orenstein, W.B. Sanders Company, 1999; *Remington's Pharmaceutical Sciences 20th Edition*, Ed. Gennaro, Mack Publishing, 2000; and *Modern Pharmaceutics 2nd Edition*, Eds. Banker and Rhodes, Marcel Dekker, Inc., 1990, each of which are hereby incorporated by reference herein.

Pharmaceutically acceptable carriers facilitate storage and administration of an immunogen to a patient. Pharmaceutically acceptable carriers may contain different components such as a buffer, sterile water for injection, normal saline or phosphate buffered saline, sucrose, histidine, salts and polysorbate.

Immunogens can be administered by different routes such as subcutaneous, intramuscular, or mucosal. Subcutaneous and intramuscular administration can be performed using, for example, needles or jet-injectors.

Suitable dosing regimens are preferably determined taking into account factors well known in the art including age, weight, sex and medical condition of the patient; the route of administration; the desired effect; and the particular compound employed. The immunogen can be used in multi-dose vaccine formats. It is expected that a dose would consist of the range of 1.0 μg to 1.0 mg total polypeptide, in different embodiments of the present invention the range is 0.01 mg to 1.0 mg and 0.1 mg to 1.0 mg.

The timing of doses depends upon factors well known in the art. After the initial administration one or more booster doses may subsequently be administered to maintain or boost antibody titers. An example of a dosing regime would be day 1, 1 month, a third dose at either 4, 6 or 12 months, and additional booster doses at distant times as needed.

Generation of Antibodies

An ORF0594 related polypeptide able to induce protective immunity can be used to generate antibodies and antibody fragments that bind to the polypeptide or to *S. aureus*. Such antibodies and antibody fragments have different uses including use in polypeptide purification, *S. aureus* identification, or in therapeutic or prophylactic treatment against *S. aureus* infection.

Antibodies can be polyclonal or monoclonal. Techniques for producing and using antibodies are well known in the art. Examples of such techniques are described in Ausubel, *Current Protocols in Molecular Biology,* John Wiley, 1987-1998, Harlow *et al., Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988, and Kohler *et al., Nature 256*:495-497, 1975.

EXAMPLES

Examples are provided below further illustrating different features of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

Example 1: Use of a SEQ ID NO: 1 Region to Provide Protective Immunity

This example illustrates the ability of a SEQ ID NO: 1 polypeptide region to provide protective immunity in an animal model. The SEQ ID NO: 1 region was part of a His-tagged polypeptide having the amino acid sequence of SEQ ID NO: 3.

ORF0594 Cloning and Expression and Modification

An ORF0594 DNA sequence was translated using Vector NTI software and the resulting 1243 amino acid sequence was analyzed. PCR primers were designed to amplify the gene starting at the first asparagine residue and ending prior to the stop codon at the terminal asparagine residue. These PCR primers also had additional NcoI (forward primer) and XhoI (reverse primer) sites to facilitate cloning into the expression vector.

The protein was designed to be expressed from the pET28 vector with the terminal His residues and the stop codon encoded by the vector. In addition, a glycine residue

was added to the protein after the methionine initiator. The resulting amplified (3735bp) DNA sequence encodes a 1245 amino acid mutated form of mature ORF0594.

PCR amplified sequences were ligated into the pET28 vector (Novagen) using the NcoI/XhoI sites that had been engineered into the PCR primers and introduced into *E. coli* DH5α (Invitrogen) by heat shock. No colonies were obtained.

The protein was further analyzed and non-identical repeat regions were identified at the Carboxyl-terminus. PCR primers were designed to amplify a completed non-identical repeat. Additional amino and carboxyl terminal sequences were also included so as to help the protein achieve a native confirmation.

PCR amplified sequences were ligated into the pET30 vector (Novagen) using the NcoI/XhoI sites that had been engineered into the PCR primers and introduced into *E. coli* DH5α (Invitrogen) by heat shock. Colonies were selected, grown in LB with 30 μg/mL kanamycin, DNA minipreps made (Promega), and insert integrity determined by restriction digestion and PCR. Four minipreps with correct insert size were sequenced using the primers listed in Table 1. A clone was selected containing no DNA changes from the desired sequence. The resulting clone expressed in the pET30 vector (Novagen) contains an N-terminal His tag with 46 residues and a stop codon encoded by the vector.

Table 1

SEQ ID NO:	Description	Sequence
6	ORF594F	ACCGGTTCCATATGAGAGATAAGAAAGGACCGGT
7	ORF594R	CCGGCGGCCCTCGAGATTCTTTCTTCTACGAGCCAATAAC
8	ORF594-AF	GAGATATACCATGGGCAGAACTGACTTGAAAGGTTCAGA
9	ORF594-AR	CCGGCGGCCCTCGAGTTATGTCGTTATTGTCTTCTCACCT

E. coli HMS174(DE3) cells (Novagen) were transformed and grown on LB plates containing kanamycin (30ug/ml); 3 colonies were selected for expression testing. Liquid LB (kanamycin) cultures were incubated at 37°C, 250 rpm until the A_{600} was between 0.6 and 1.0 and then induced by the addition of IPTG to final concentrations of 1 mM followed by three hours further incubation. Cultures were harvested by centrifugation at 5000 x g for 5 minutes at 4°C. Cells were resuspended in 500 μl lysis buffer (Bug Buster, with protease inhibitors, Novagen). An equal volume of loading buffer (supplemented with β-mecapto ethanol to 5% final volume) was added prior to heating the samples at 70°C for 5 minutes. Extracts were run on Novex 4-20% Tris-Glycine gels and assayed for protein (Coomassie Blue stained) and blotted onto nitrocellulose and probed with anti-HIS6 antibodies (Zymedd).

Polypeptide Purification

Recombinant *E. coli* cells (39 grams wet cell weight) were suspended in Lysis Buffer (50 mM sodium phosphate, pH 8.0, 0.15 M NaCl, 2 mM $MgCl_2$, 10 mM imidazole, 0.1% Tween™-80, and 0.02% sodium azide) at 3.7 ml per gram of cell wet weight. Protease Inhibitor Cocktail for use with poly-(Histidine)-tagged proteins (Roche #1873580) was added to the suspension at 1 tablet per 15 grams of cell paste. Benzonase™ (EM Ind.) was added to 1 μL/mL. Cell lysis was accomplished by passing the suspension through a microfluidizer at 14,000 PSI (Microfluidics Model 110S) three times. The cell suspension was cooled on ice between each pass so that the temperature remained below 25°C. Cell debris was pelleted at 11,000 x g for 30 minutes at 4°C, and the supernatant retained.

Proteins bearing a His-tag were purified from the supernatant. The supernatant was mixed with 12 mL of Ni^+-NTA agarose (Qiagen) at 4°C with gentle inversion for 18 hours. The mixture was poured into an open column (1.5 cm x 20 cm) and the non-bound fraction was collected in bulk. The column was washed with Wash Buffer (50 mM sodium phosphate, pH 8.0, 0.3 M NaCl, 20 mM imidazole, and 0.1% Tween™-80). His-tagged protein was eluted with a step gradient of 300 mM imidazole, 20 mM Tris-HCl, pH 8, 0.3 M NaCl, 0.1% Tween™-80.

Fractions containing ORF0594 protein were detected by Coomassie stained SDS-PAGE and pooled. Pooled fractions were filtered through a 0.2 micron filter to remove particulate material, and were applied on a size-exclusion column (Sephacryl S-300 26/60 column, Amersham Biosciences) and eluted at 1 mL/min with 30 mM MOPS pH 7.0, 0.3 M NaCl, and 10% glycerol. Fractions containing ORF0594 were detected by Coomassie stained SDS-PAGE and Western blotting (anti-tetra His Mab, Qiagen). Protein was determined by BCA (Pierce). Purity was determined by densitometry of Coomassie stained gels.

Preparation of S. Aureus Challenge

S. aureus was grown on TSA plates at 37°C overnight. The bacteria were washed from the TSA plates by adding 5 ml of PBS onto a plate and gently resuspending the bacteria with a sterile spreader. The bacterial suspension was spun at 6000 rpm for 20 minutes using a Sorvall RC-5B centrifuge (DuPont Instruments). The pellet was resuspended in 16% glycerol and aliquots were stored frozen at –70°C.

Prior to use, inocula were thawed, appropriately diluted and used for infection. Each stock was titrated at least 3 times to determine the appropriate dose inducing slow kinetics of death in naive mice. The potency of the bacterial inoculum (80 to 90% lethality) was constantly monitored to assure reproducibility of the model. Ten days before each challenge

experiment, a group of 10 control animals (immunized with adjuvant alone) were challenged and monitored.

Protection Studies for a SEQ ID NO: 2 Polypeptide

Twenty BALB/c mice were immunized with three doses of a SEQ ID NO: 2 polypeptide (20 μg per dose) on aluminum hydroxyphosphate adjuvant (450 μg per dose). Aluminum hydroxyphosphate adjuvant (AHP) is described by Klein *et al., Journal of Pharmaceutical Sciences 89*:311-321, 2000. The doses were administered as two 50 μl injections on days 0, 7 and 21. The mice were bled on day 28, and their sera were screened by ELSIA for reactivity to the SEQ ID NO: 2 polypeptide. On day 35 of the experiment the mice were challenged by intravenous injection of S. aureus grown at a dose (10^8 CFU ml). The mice were monitored over a 10 day period for survival.

Two different sets of experiments were performed to measure the ability of a SEQ ID NO: 1 related peptide to provide protective immunity. In the first experiment, an immunogen of SEQ ID NO: 4 in AHP was used as a positive control. The positive control was administered at 10 μg per dose in the same manner as the SEQ ID NO: 2 polypeptide. A second experiment was performed without a positive control. The results of the both experiments are shown in Figure 3.

The employed animal model is very extreme due to the high dose of bacteria administered and the size of the animal, and has some variability. In human disease, infection is initiated by a reduced bacterial load. Modest protection of immunogen with the employed animal model provides support for the effectiveness of a vaccine in humans.

In the first experiment, 7 mice from the SEQ ID NO: 2 polypeptide immunized group survived compared to 3 surviving in the AHP control group and 11 surviving from the SEQ ID NO: 4 immunized group. The results from the first experiment indicate that SEQ ID NO: 2 polypeptide is moderately protective.

In the second experiment, an equal number of mice survived that were immunized with the SEQ ID NO: 2 polypeptide compared to mice surviving in the AHP group. However, the second experiment did not contain a positive control. Thus, the results of the second experiment are suspect compared to the first experiment.

Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.